

Autoradiographs prepared from two-dimensional paper chromatograms (solvent systems: isopropanol-ammonia-water and n-butanol-acetic acid-water) of the neat urine again revealed three major spots corresponding in  $R_F$  values to 5-HTP, 5-HIAA, and serotonin. These results, obtained with untreated patients, are similar to those obtained with the BAS-treated patients; obviously the action of BAS on monoamine oxidase cannot be the explanation.

A comparison of the ability of BAS and iproniazid to block the conversion of 10 mg of serotonin to urinary 5-HIAA, administered intraperitoneally to rats, indicated that iproniazid at 100 mg/kg effectively blocked monoamine oxidase, whereas BAS up to 200 mg/kg did not. Similarly, the formation of endogenous 5-HIAA in the rat was blocked by iproniazid but not by BAS.

Zeller (2) has shown that BAS is not a monoamine oxidase inhibitor. Evidence we have obtained since our initial publication (1) indicates that Zeller is right and that we misinterpreted our earlier data (3, 4).

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#### References and Notes

1. A. Feldstein, H. Hoagland, H. Freeman, *Science* 130, 500 (1959).
2. E. A. Zeller, *Science*, this issue.
3. We wish to thank Marilyn George and William Connors for technical assistance. We also wish to acknowledge grants from the Ford Foundation and the Scottish Rite Committee on Research in Schizophrenia.
4. S. Spector, P. A. Shore, and B. B. Brodie, [*Science* 132, 735 (1960)] have also noted that BAS is not a monoamine oxidase inhibitor. Their article appeared after we submitted for publication this reply to Zeller, and our answer also applies to their report.

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### Virus Isolated from Inclusion Conjunctivitis of Newborn (Inclusion Blepharitis)

**Abstract.** An infant developed acute conjunctivitis 7 days after birth. Smears of the mucopurulent discharge contained many typical elementary-body inclusions. From conjunctival scrapings a virus resembling trachoma was grown in eggs. When instilled into monkey eyes, it produced an acute conjunctivitis resembling the human disease. Nine other patients with inclusion conjunctivitis of similar intensity failed to yield viruses.

Inclusion conjunctivitis is an acute nonbacterial eye disease most commonly observed in newborn children. It begins in the first 2 weeks of life

with redness, edema, infiltration of the conjunctiva, and a mucopurulent exudate. It does not involve the cornea, and the conjunctiva heals spontaneously in weeks or months without scarring. With the application of sulfonamide or tetracycline drugs to the conjunctiva, the infection regresses in a few days. Fifty years ago it was shown that in smears from infected conjunctiva some epithelial cells contained inclusions resembling those of trachoma (1). Similar inclusions were demonstrated in epithelial cells from the mother's cervix (2). While it is evident that in the newborn the infection is acquired from the mother's genital tract, direct-contact transmission to the adult eye is occasionally observed (3).

The epithelial cell inclusions, composed of elementary or initial bodies, are morphologically similar to those of the psittacosis-lymphogranuloma venereum-trachoma group of viruses. However, in spite of the profusion of viral particles seen in smears from inclusion conjunctivitis of the newborn, the virus has not been grown in many attempts made since 1910. When a successful method for growing trachoma viruses became available (4), it was soon applied to studies on inclusion blennorrhoea, and a successful isolation of such a virus was reported (5). During the past 2 years we have been engaged in growing trachoma viruses from patients in the United States and studying the characteristics of these viruses (6, 7). We included in this study ten newborn children with the clinical and microscopic diagnosis of inclusion conjunctivitis. It is the purpose of this communication to report the first isolation of an inclusion conjunctivitis virus in the United States and to confirm and extend the observations of Jones *et al.* (5, 8).

The infant, a normal full-term boy, developed redness and discharge of the right eye 7 days after birth. Three days later the left eye became involved. When treatment with boric acid drops had no effect, the child was examined (on the 16th day of life) by one of us (D.G.V.). There was hyperemia of the upper and lower palpebral conjunctivae, and there was a moderate amount of mucopurulent discharge. The cornea was clear. Conjunctival smears showed many neutrophils and some lymphocytes and monocytes; at least 10 percent of the epithelial cells contained typical inclusions in all stages of development. There were also many scattered free elementary bodies, but no bacteria. After treatment with sulfisoxazole ointment, the boy recovered promptly.

Conjunctival scrapings obtained on the 12th day after onset were sus-

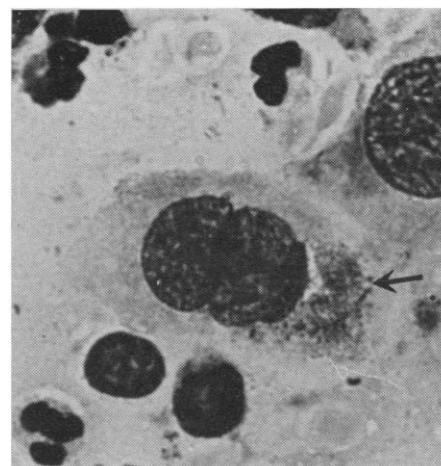


Fig. 1. Conjunctival smear from *Cynomolgus* monkey on the 8th day after infection of the eye with virus. The arrow points to an elementary-body inclusion in an epithelial cell (Giemsa stain).

ended in broth-saline containing 10 mg of streptomycin and 0.1 mg of polymyxin B per milliliter; the suspension was kept on ice for 2 hours, inoculated into the yolk sacs of 7-day embryonated eggs, and incubated at 35°C. Blind passage of yolk-sac suspensions was carried out at 7-day intervals, as described elsewhere (7). In the fifth passage some eggs died, and yolk-sac smears revealed many free elementary bodies with Giemsa or Macchiavello's stains. In the ninth passage the virus was well established with an egg LD<sub>50</sub> of 10<sup>-6.7</sup>. On two occasions the virus was reisolated from stored frozen yolk-sac material of first and third passage. Six and seven passages, respectively, were necessary before smears from eggs that had died revealed abundant visible virus. In morphology, infectivity for eggs, behavior toward antibiotics, and serologic reaction with antipsittacosis serum, the virus appeared to be indistinguishable from trachoma viruses isolated in this laboratory (6, 7).

Two *Cynomolgus* monkeys and one baboon were infected by instilling 0.2 ml of a 10<sup>-1</sup> dilution of ninth-passage yolk-sac suspension into each eye. On the 5th day these animals developed intense conjunctival hyperemia, resulting in bleeding upon light touch. There was a mucopurulent exudate consisting largely of neutrophils and lymphocytes, and some epithelial cells contained typical inclusions (Fig. 1) indistinguishable from those seen in the newborn infant. Later the monkeys developed conjunctival infiltration and follicles which persisted for over 3 weeks. On the 12th day of infection, virus was reisolated from a conjunctival scraping of a *Cynomolgus* monkey by egg inoculation. An attempt to adapt

the virus to mouse brain (9) was unsuccessful.

Conjunctival scrapings from nine other, similar infants with inclusion conjunctivitis treated in an identical manner failed to yield virus. This fact, and the high number of egg passages required for demonstration of viral activity in the one infant yielding virus (five, six, and seven passages on three attempts) suggest that of the vast number of virus particles seen microscopically in conjunctival smears only a minute proportion was able to propagate in eggs. During the period of these isolation attempts trachoma viruses proliferated readily in eggs from the same source. Thus, seasonal insusceptibility of eggs (7) is not a likely explanation for the failure of virus isolation in nine out of ten patients.

Undoubtedly the mother's genital tract is the source of the newborn's infection with inclusion conjunctivitis (3). The mother of our patient had marked vaginal discharge late in pregnancy, and examination 10 weeks after delivery indicated resolving cervicitis. However, cervical scrapings yielded no epithelial inclusions, and gross bacterial contamination vitiated attempts at virus isolation.

Whereas trachoma regularly involves the cornea and, if untreated, tends to produce progressive eye-tissue changes, inclusion conjunctivitis in newborn or adult does neither. We are currently comparing strains of trachoma virus (7) with the strain of inclusion conjunctivitis virus, in the hope of demonstrating some biological difference which might parallel the evident differences in the diseases caused by these agents.

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#### References and Notes

1. K. Stargardt, *Arch. Ophthalmol., Graefe's* **69**, 524 (1909); L. Schmeichler, *Berlin. klin. Wochschr.* **46**, 2055 (1909).
2. L. Halberstaedter and S. von Prowazek, *Berlin. klin. Wochschr.* **46**, 1839 (1909).
3. P. Thygeson, *Am. J. Ophthalmol.* **17**, 1019 (1934).
4. F. F. Tang, H. L. Chang, Y. T. Huang, K. C. Wang, *Chinese Med. J.* **75**, 429 (1957).
5. B. R. Jones, L. H. Collier, C. H. Smith, *Lancet* **1**, 902 (1959).
6. L. Hanna, P. Thygeson, E. Jawetz, C. Dawson, *Science* **130**, 1339 (1959).
7. L. Hanna, E. Jawetz, P. Thygeson, C. Dawson, *Proc. Soc. Exptl. Biol. Med.* **104**, 142 (1960).
8. This work was supported in part by grants from the National Institutes of Health (B 604), the Burroughs Wellcome Fund, and the Research Committee of the University of California.
9. W. E. Hurst and P. Reeve, *Nature* **186**, 336 (1960).

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2 DECEMBER 1960

## Performance Record of a Parthenogenetic Turkey Male

**Abstract.** A Beltsville Small White turkey poult of parthenogenetic origin hatched in the spring of 1958, matured, and produced semen containing viable spermatozoa. Semen from this male was used in January 1959 to inseminate seven virgin and seven previously mated Beltsville Small White turkey hens. Three hundred and twenty eggs were incubated, of which 175 or 54.7 percent were infertile. One hundred and twenty-two poults, about equally divided as to sex, hatched unaided from 145 fertile eggs.

During 1958 more than 8000 unfertilized eggs from 214 Beltsville Small White turkey hens were incubated, and data were collected on the incidence of parthenogenetic development. Seven hundred and twenty-two of these eggs (9.0 percent) were found to contain embryos of various ages, including 20 which survived to 29 days of incubation and were helped from the shell. One of three parthenogenetic poults raised to maturity produced usable quantities of semen containing viable spermatozoa. Semen from this parthenogenetic male was used in January 1959 to inseminate 14 Beltsville Small White hens, seven of which were young, unselected virgins. The other seven hens from the parthenogenetic line had been mated 8 months prior to these tests. Eggs laid by these 14 hens were identified as to hen number and subsequently incubated to obtain data on fertility and hatchability.

Data presented in Table 1 show that infertility was generally higher than would be expected for eggs from regular matings of Beltsville Small White turkeys, amounting to 50.3 percent of total eggs for the virgins and 61.1 percent for previously mated hens. Hatchability, when calculated on the basis of fertile eggs, was satisfactory, amounting to 85.1 percent for the virgins and 82.4 percent for eggs of previously mated hens. These percentages are within the range of normal variation for eggs of mated flocks of these turkeys.

Early embryonic mortality, 8.5 percent for virgins and 13.7 percent for previously mated hens, was generally higher than that for unhatched eggs from normal flocks of Beltsville Small White turkeys. The percentages of late mortality—6.4 percent for virgins, 3.9 percent for previously mated hens—may be considered normal, certainly no higher than normal. Late embryonic mortality in eggs from regular matings is generally two or three times greater than that occurring during the first 7 days of incubation.

One hundred and twenty-two poults were hatched from the 147 fertile eggs produced by the 14 hens. These poults

Table 1. Incubation record of eggs produced by 14 Beltsville Small White turkey hens after insemination with semen from a parthenogenetic male.

Item	Virgins		Previously mated	
	No.	%	No.	%
Hens inseminated	7		7	
Eggs laid following insemination	189		131	
Fertile eggs	94	49.7*	51	38.9*
Dead embryos (1-14 days)	8	8.5	7	13.7
Dead embryos (15-28 days)	6	6.4	2	3.9
Poults hatched	80	85.1	42	82.4

\* Percentage based on total eggs (the other percentages are based on fertile eggs).

were relatively free of major anatomical defects and thus were able to hatch unaided. They were about equally divided with respect to numbers of males and females. Poults were hatched from eggs laid as many as 44 days previously, and fertile eggs were obtained for as long a time as 50 days following a single insemination. The duration of fertility on the part of the sperm of the parthenogenetic male compares favorably with duration of fertility in normal turkeys as given in previously published figures.

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## Thermodynamic Treatment of Radio-Tracer Movements across Biological Membranes

**Abstract.** The movements of radioactive tracers across living cell membranes are discussed on the basis of thermodynamics of irreversible processes. Krogh's equation describing the flux of a tracer as a function of time is derived, and the significance of the "permeability" constant is clarified.

It is well known that, when a living cell is immersed in a large volume of medium containing a radioactive tracer, the intracellular concentration of the tracer rises roughly exponentially with time. The final concentration of the tracer is determined, as is expected, by the ratio at which the nonradioactive species of the same chemical substance is distributed across the cell membrane. The time constant with which the intracellular tracer concentration rises is considered to be determined by the "permeability" of the membrane with respect to the substance (*1*). The purpose of this report is to treat this behavior of the tracer movement from the